*Acta med. nagasaki.* 8 : 94–109

# The Experimental Study on Organ Lipoprotein Lipase, Especially in Relation to the Lung

Hisashi YAMADA*

*Second Department of Internal Medicine,*
*Nagasaki University School of Medicine,*
*Nagasaki, Japan*

Received for publication March 5, 1964

Determination of the lipolytic activity in various organs of rats were done by means of organ homogenate in vitro, the significance of the lipolytic activity was more characterized by the inhibition study of the Lipoprotein Lipase (LPL). The activity of the lung tissue was high followed by the cardiac muscle or adipose tissue. After force feeding fat meal or after heparin administration in vitro, the significant elevation was observed. And also after carbon tetrachloride poisoning or aseptic pneumonia induced by silver nitrate solution injection, the lipolytic activity in the lung increased with the endogeneous lipid transportation from the depot fat to the liver tissue. The histological character of the lung tissue is rich in arterioles and capillaries and also rich in mast cells and the lung has a anatomical speciality; the first organ to be approached by exogeneous lipid. These findings suggest that the lung is one of the organs which produce LPL and may display some role in the exogeneous and endogeneous lipid transportation.

In 1943 HAHN[1] discovered the rapid clearing of alimentary lipemia in dogs by the injection of heparin, and in 1950 Anderson and Fawcett[2] demonstrated that plasma obtained after heparin injection contained a factor which cleared lipemia in vitro.

Many studies[3][4][5][6][7] on clearing factor were achieved in the past 20 years and the clearing factor was proved to be a new enzyme, which was neither esterase, pancreatic lipase nor tributylinase. Although the clearing factor has not yet been obtained in pure form, its action has been definite to catalyse the hydrolysis of triglyceride moiety of chylomicrons and low density lipoproteins, because free fatty acid was liberated in plasma during the clearing of the postalimental lipemic plasma.

On the other hand, studies on the Lipoprotein Lipase (LPL) activity in the tissue[8][9][10][11] were at first undertaken in adipose tissue concerning a nutritional state or a neurohumoral disturbances, and in cardiac

* 山 田　　久

muscle[12)13)14)] which takes up free fatty acid as the energy source.

Although there are many difficulties in method to determine LPL activity of the tissue, LPL in plasma and in tissue would be identical by the theoretical point of view[12)].

It is generally believed that LPL plays a important role in transfer of fat from the circulating blood into a peripheral tissues[15)16)].

It has been supposed by some investigators that the disturbance of the lipid metabolism might happen in pulmonary diseases[17)18)], because lipemia has been often observed clinically in pneumonia and lung cancer, and fatty liver has been recognized in autopsies of pulmonary tuberculosis[19)].

Also, the lung is anatomically positioned as the first organ to be approached from the exogeneous fat.

These facts would be sufficient to suggest the close relationship between the lung and neutral lipid transportation in lipid metabolism.

In this study, two experiments on LPL activity were undertaken for the resolution of these problems. The first was the improvement of the LPL assay-method because the leading method has been insufficient in sensitivity to detect it in tissues and the second was to evaluate the LPL activity in lung comparing with adipose tissue, liver, heart and kidney which were thought to be the most active organ in lipid metabolism, especially in lipoprotein and non-esterified fatty acid (NEFA) metabolism.

## I. Experiment for Confirmation of LPL Activity Assay in Organ.

### Materials and Method

*Preparation of materials;* Wister-strain male albino rats from 180 to 250 g body weight, were used. The rats were fed with regular stock diet and water ad libitum until the biginning of the experiment. The experimental animals were fasted overnight before the experiment, and were anesthetized with 5 mg/100g body weight sodium pentobarbital and then they were killed by decapitation. Each organ was immediately removed, and washed with physiological saline solution. Each tissue, weighing exactly 500 mg was placed into the glass homogenizer with 2 ml of 0.9% physiological saline solution. After homogenizing them in the cold, the homogenate was centrifuged for 10 minutes at 3000 rpm in 0°C, the supernatant layer was separated, and each ml of them was supplied to the measurement of lipolytic activity (LA).

*Assay of lipolytic activity;* The substrate consisted of 0.1 vol. of 20 % Fatgen (sesame oil emulsion), 1 vol. of dogs oxalated plasma (pooled from several dogs, heated at 56°C for 10 minutes, immediatly cooled, centrifuged to remove the fibrin and stored at −20°C), 1 vol. of 5% bovine albumin and 2 vol. of tris-hydroxymethyl aminomethane buffer (pH 7.7). The substrate was preincubated at 37°C for 30 minutes just before use.

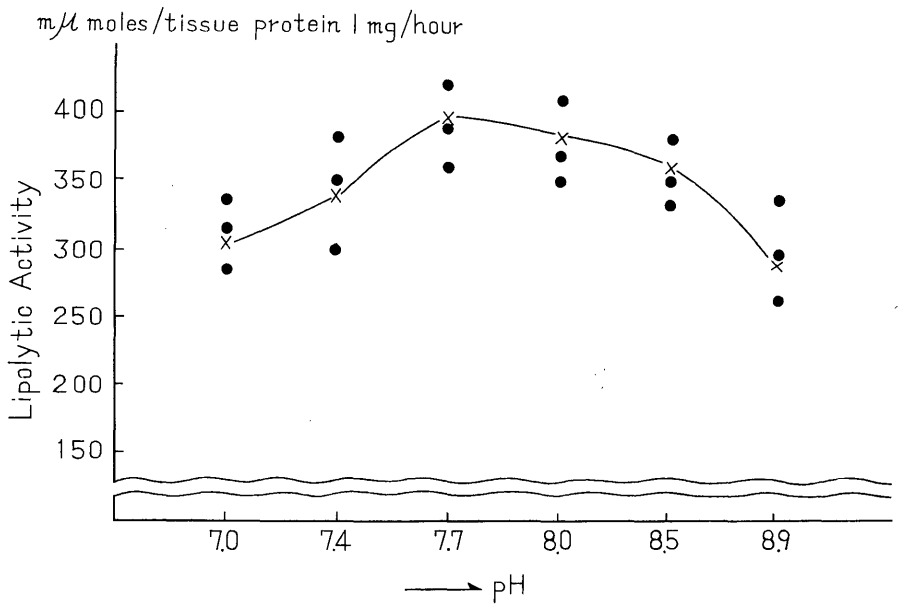
Immediately after mixing 1 ml of the sample into 2 ml of substrate, 1 ml of the mixture was pipetted into Dole's[20] extraction mixture and remaining mixture was continued to incubated at 37°C for 60 minutes. Thereafter 1 ml of the mixture was also pipetted into the Dole's mixture.

NEFA was measured by Dole's method[20]. Namely, 1 ml of sample are added into a glass-stoppered test tube with 5 ml extraction mixture and shaken for 10 minutes. And then 3 ml heptane and 2 ml water are added and the mixture are shaken again for 5 minutes. To a final tube containing 1 ml Dole's titration mixture is transferred 3 ml of the heptane layer and it is then titrated with 0.018 N sodium hydroxide while being agitated with a stream of nitrogen. The heptane layers from appropriate titration blanks and palmitic acid standards are similarly titrated, in every experiment. The differences of NEFA concentration in the mixture before and after the incubation were calcurated. The protein concentration in the homogenate was determined by Biuret's method, and then the unit of activity was expressed mμ moles of linoleic acid formed per hour per mg protein.

## Results

The effect of pH in the reactions medium were observed. The lipolytic activity was measured by using the lung homogenate in the following varing pH of the substrate, pH 7.0, 7.4, 7.7, 8.0, 8.5 and 8.9. The results were shown in Fig. 1.

**Fig. 1.** Effects of pH on the Lipolytic Activity

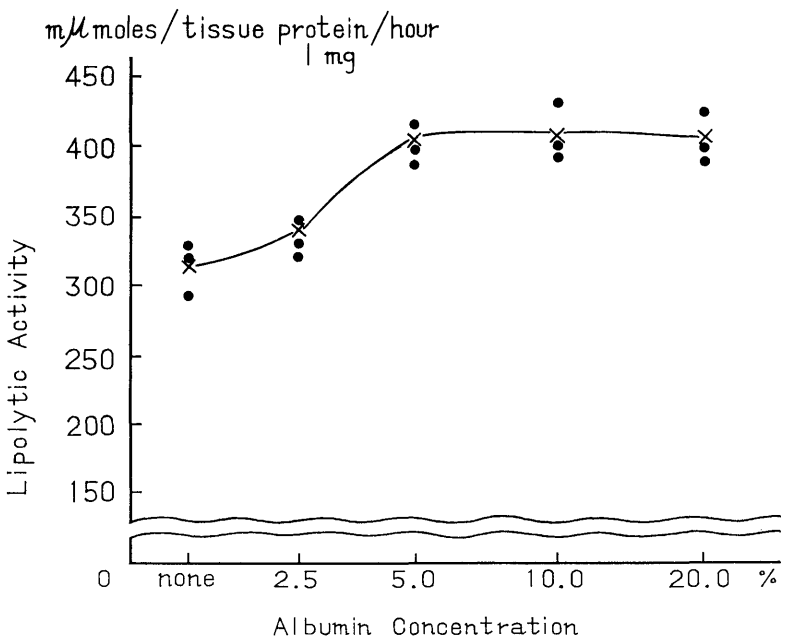


These observations suggest that pH 7.7 is optimum for the measurement of the lipolytic activity.

As a fatty acid acceptor in the reaction mixture, the bovine albumin solution were used, and the influence of albumin concentration was observed.

The lipolytic activities were measured by using the lung homogenate in the following varing the concentration, 2.5%, 5.0%, 10.0%, 20.0% and 0.9% physiological saline solution, instead of albumin solution. The results were shown in Fig. 2.

Fig. 2




It seems that 5% albumin solution in the reaction mixture is quite enough as the fatty acid acceptor.

When dog plasma was used as a part of the substrate, an influence of the inhibitor must be taken into consideration. B. HOOD[21], G. ANGERVALL[22][23], F. C. MONKHOUSE and P. G. MACKNESON[24] had studied the inhibitor in plasma which was removed by heating the plasma in 56°C. This problem was examined. Postheparin plasma was used as a lipolytic enzyme source. (The dog blood was collected at 10 minutes after an intravenous injection of 100 units of heparin per 100 g of body weight, and plasma was obtained by centrifuging the blood for 10 minutes at 3000 rpm in cold.)

**Fig. 3.** Effects of heating at 56°C for varing lengths of time on pools of dog plasma.

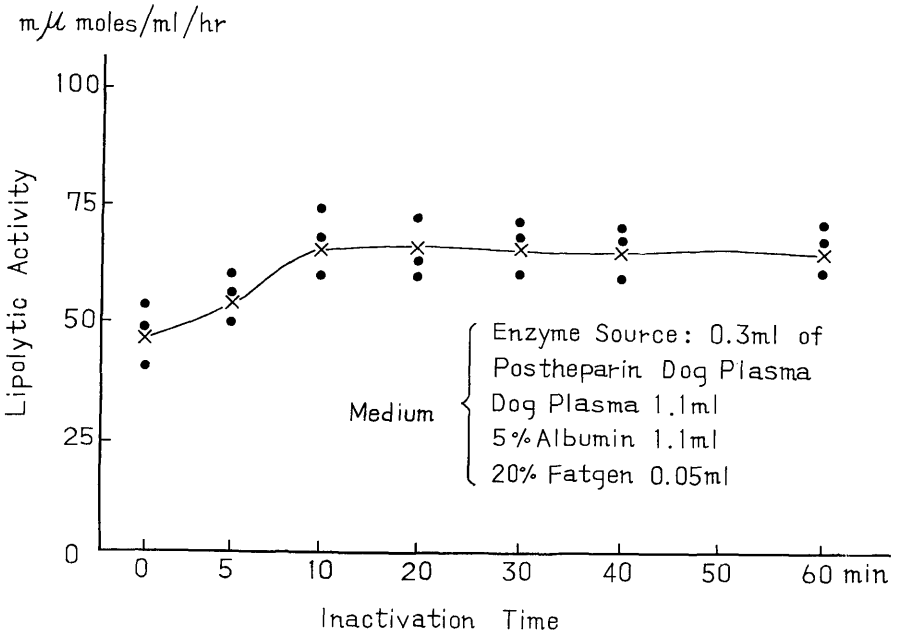
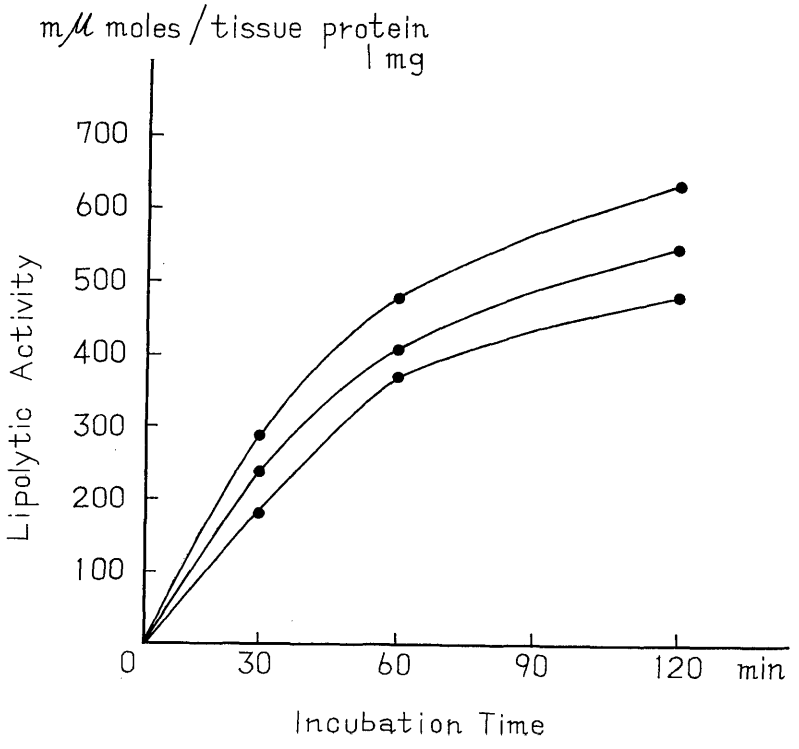



**Fig. 4.** Effects of Incubation Time on the Lipolytic Activity.



Heating 10 minutes appears optimum, therefore, the dog plasma used for the substrate was heated in a water bath at 56°C for 10 minutes, cooled immediately, centrifuged, and stored at −20°C.

Incubation time: Using lung homogenate as the lipolytic enzyme source, the lipolytic activity was measured in various incubation time.

As the results shown in Fig. 4, approximately quadratic curve was obtained. This curve shows that the amount of the lipolytic activity in the first 60 minutes was larger than that in the next 60 minutes.

Discussion

The pH in the medium, the concentration of albumin solution added to the substrate as NEFA acceptor, and the incubation time were examined and the optimal conditions in this enzymatic reaction system were obtained. High level of the lipolytic activities were detected in pH 7.7 which is in close agreement with the results of G. Murakami and others. The object for the NEFA acceptor was attained completely by adding 5% albumin solution to the medium. It is agreeable to the results of D. S. Robinson and others that the increase rate of NEFA was linear in the first 60 minutes incubation.

When G. Angervall, B. Hood[21)22)23)] and others studied on the lipemia clearing enzyme in plasma of the atherosclerotic patients and hypercholesterolemic patients, they discovered an inhibitory effect in the post-heparin plasma on the clearing phenomenon. And thereafter the details of this inhibitory factor was studied by several investigators. F. C. Monkhouse[24)] and others examined exactly the inhibitor which inhibits the release of NEFA from the chylomicrons in plasma, and they pointed out the variation of inhibitory effects in species and individuals.

In this experiment of LPL assay in tissues, the dog oxalated plasma was used in the substrate, and the inactivation of inhibitors in the plasma were done by means of heating them at 56°C in water bath according to the techinique of Monkhouse. And the affection of this treatment on the lipolytic activity was detected.

The results above mentioned, emphasized that it is better to use the inhibitor free plasma when dog plasma is used as substrate, because the influence of the inhibitor is so great as approximately one half time of it leading.

II. Experiment for Evaluation of LPL Activity in various Organs especially in Lung.

Treatment of Animals.

The animals, which were supplied in this experiment, were in similar condition to the previous experiment and divided into three groups.

1) Six rats were injected 0.25ml/100g body weight of carbon tetrachloride into the gluteus muscle, and other five rats were fed 0.5

ml/100g body weight of a mixture consisting 1 vol. of carbon tetrachloride and 1 vol. of olive oil. Force-feeding was done through a stomach tube under sodium pentobarbital anaesthesia (intraperitoneal injection of 5 mg/100g body weight). Sixteen hours after carbon tetrachloride administration, these rats were killed by decapitation.

2) Eight rats were force-fed 0.5 ml/100g body weight of olive oil. Four rats were killed after 3 hours and other four rats after 5 hours by decapitation.

3) Five rats were administered with 0.05 ml of 1% silver nitrate solution by intratracheal injection and killed after 24 hours. At this time, the findings of aseptic pneumonia were seen macro- and microscopically.

Immediately after autopsy of these 3 type treated rats, lung, adipose tissue, liver, kidney and heart were removed for the determination of lipolytic activity.

Results

1) The lipolytic activity of various organs in intact rats. Using the above described techinique, the LA of the lung, liver, heart, kidney and adipose tissue in rats (8 examples) were measured.

The results are shown in Table 1. The lipolytic activities of the heart, lung, and adipose tissue were significantly greater than those of the liver and kidney.

Table 1.
Lipolytic Activity in Various Organs of Intact Rats.

| Organ | Lung | Liver | Heart | Adipose tissue | Kidney |
|---|---|---|---|---|---|
| No. 1 | 483 | 283 | 570 | 534 | 294 |
| 2 | 405 | 238 | 498 | 453 | 356 |
| 3 | 424 | 256 | 526 | 434 | 264 |
| 4 | 392 | 300 | 601 | 505 | 202 |
| 5 | 368 | 344 | 501 | 537 | 244 |
| 6 | 434 | 273 | 566 | 474 | 224 |
| 7 | 455 | 242 | 483 | 521 | 238 |
| 8 | 466 | 307 | 541 | 514 | 228 |
| Mean value | 427 | 279 | 534 | 497 | 257* |
| ± S. D. | ±35 | ±31 | ±39 | ±36 | ±46 |

* Lipolytic Activity is expressed as mμ moles/tissue protein 1 mg/hour.

2) The lipolytic activity after fat administration.

Eight rats were force-fed with olive oil by stomach tube, and were divided into two groups (4 : 4). The first group were killed after 3 hours and the second group 5 hours after force-feeding. In both groups

the lipolytic activity of the lung, adipose tissue and heart were markedly increased, ($P < 0.01$) but that of the liver and kidney changed insignificantly. In comparing these two groups, changes of the LA of the organs were more marked in the first group. (See Table 2)

Table 2.
Lipolytic Activity after force-feeding of Olive Oil by Stomach Tube.

| Organ | Lung | Liver | Heart | Adipose tissue | Kidney |
|---|---|---|---|---|---|
| No. 1 | 672 | 299 | 556 | 778 | 308 |
| 2 | 491 | 292 | 688 | 702 | 322 |
| 3 | 629 | 310 | 620 | 921 | 280 |
| 4 | 660 | 296 | 626 | 756 | 290 |
| Mean value | 612 | 299 | 623 | 790 | 301* |
| ± S .D. | ±71 | ±69 | ±48 | ±79 | ±18 |
| 5 | 534 | 316 | 547 | 794 | 312 |
| 6 | 463 | 275 | 588 | 836 | 273 |
| 7 | 481 | 327 | 533 | 609 | 301 |
| 8 | 575 | 261 | 510 | 664 | 329 |
| Mean value | 510 | 296 | 544 | 726 | 304* |
| ± S. D. | ±44 | ±28 | ±27 | ±93 | ±21 |

* Lipolytic Activity is expressed as mμ moles/tissue protein 1 mg/hour.
♯ No. 1 ~ No. 4 : 3 hours after the force-feeding.
No. 5 ~ No. 8 : 5 hours after the force-feeding.

3) Effect of Heparin Administration in vitro.

The lipolytic activity of each organ in intact rats were measured by adding heparin into the reaction mixture. The results were given

Table 3.
Effects of Heparin added in vitro on the Lipolytic Activity. ♯

| Organ | Lung | Liver | Heart | Adipose tissue | Kidney |
|---|---|---|---|---|---|
| No. 1 | 518 | 307 | 609 | 513 | 308 |
| 2 | 532 | 443 | 548 | 561 | 287 |
| 3 | 560 | 283 | 591 | 541 | 266 |
| 4 | 518 | 256 | 587 | 569 | 280 |
| Mean value | 532 | 276 | 584 | 546 | 287* |
| ± S. D. | ±18 | ±20 | ±22 | ±20 | ±14 |

♯ 100u. of Heparin was added into the Medium.
* Lipolytic Activity is expressed as mμ moles/tissue protein 1 mg/hour.

in Table 3. Increase of the lipolytic activity in the lung ($P<0.01$) and heart ($P<0.05$) were significant. There were no significant change in other tissues.

4) Effect of various inhibitors to the lipolytic activity in each organ.

Hitherto, the LA of organs were measured with these homogenate. It was thought that the tissue contains lipoprotein lipase and many other esterases and these enzyme would display lipolytic activity.

Twenty mg protamine sulfate, 1M of high density sodium chloride and 0.2 M of sodium fluoride were added to the reaction medium respectively. When protamine sulfate was added to the reaction medium, the pH of the medium dropped down to pH 6.2～6.3, so that the pH was readjusted immediately to pH 7.7～7.8 by addition of dilute sodium hydroxide. Thereafter the alteration of the lipolytic activities were observed, these results are summarized in Table 4.

The inhibitory effects on enzyme were 77.0－86.4% by protamine sulfate and 72.0－90.0% by sodium chloride in the heart, 59.0－78.8%

Table 4.

Effects of the Various Inhibitors on the Lipolytic Activity in Intact Rats

| Organ / No. of Samples | Inhibitors | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Protamine Sulfate (20mg) | | | Sodium Chloride (1M) | | | Sodium Fluoride (0.2M) | | |
| | Added | None | Inhibitory Effect in % | Added | None | Inhibitory Effect in % | Added | None | Inhibitory Effect in % |
| Lung 4 | (182 | 466 | 61.0% | (105 | 466 | 77.5% | (259 | 441 | 39.2% |
| Mean value | (112 | 410 | 72.5 | ( 67 | 410 | 84.0 | (220 | 410 | 48.2 |
| ± S. D. | (179 | 437 | 59.0 | (136 | 437 | 68.6 | (242 | 378 | 36.5 |
| | ( 77 | 364 | 78.8 | (112 | 364 | 69.0 | (228 | 448 | 49.2 |
| | | | 67.8± 8.2 | | | 74.9± 5.8 | | | 42.8± 5.1 |
| Liver 4 | (266 | 344 | 22.8 | (263 | 344 | 23.8 | (109 | 344 | 68.4 |
| Mean value | (157 | 276 | 43.3 | (185 | 276 | 32.1 | (116 | 317 | 63.7 |
| ± S. D. | (184 | 300 | 38.6 | (202 | 300 | 31.8 | (102 | 279 | 63.5 |
| | (228 | 327 | 30.2 | (279 | 327 | 14.5 | ( 96 | 327 | 70.2 |
| | | | 33.7± 8.0 | | | 25.6± 7.2 | | | 66.5± 2.9 |
| Heart 4 | ( 68 | 491 | 86.4 | ( 93 | 491 | 80.8 | (315 | 491 | 36.0 |
| Mean value | ( 50 | 565 | 84.5 | ( 65 | 565 | 88.2 | (423 | 565 | 30.0 |
| ± S. D. | (107 | 498 | 78.2 | ( 50 | 498 | 90.0 | (354 | 498 | 28.5 |
| | (111 | 484 | 77.0 | (136 | 484 | 72.0 | (330 | 484 | 31.8 |
| | | | 81.7± 3.9 | | | 82.8± 7.1 | | | 31.6± 2.8 |
| Adipose tissue 4 | ( 56 | 482 | 88.5 | ( 64 | 482 | 86.8 | (353 | 482 | 26.6 |
| Mean value | ( 92 | 445 | 70.0 | (120 | 445 | 73.0 | (297 | 465 | 36.2 |
| ± S. D. | ( 48 | 497 | 90.4 | (152 | 497 | 68.5 | (321 | 497 | 35.4 |
| | (104 | 421 | 75.2 | (121 | 421 | 70.1 | (288 | 421 | 30.5 |
| | | | 81.1±11.9 | | | 74.7± 7.2 | | | 32.2± 3.9 |
| Kidney 4 | (115 | 294 | 60.5 | (135 | 294 | 54.0 | (224 | 294 | 23.4 |
| Mean value | ( 96 | 224 | 57.0 | (148 | 224 | 34.3 | (122 | 224 | 45.0 |
| ± S. D. | (122 | 304 | 59.9 | (152 | 304 | 49.5 | (178 | 304 | 40.8 |
| | (106 | 238 | 55.5 | (109 | 238 | 54.0 | (125 | 238 | 47.2 |
| | | | 58.3± 2.1 | | | 47.9± 8.1 | | | 39.1± 7.9 |

# LA is expressed as mμ moles/tissue protein 1 mg/hour.

by protamine sulfate and 68.6−84.0 % by sodium chloride in the lung, 70.0−90.4 % by protamine sulfate and 68.5−86.8 % by sodium chloride in the adipose tissue. These organs were inhibited in high percentage. But the inhibitory effects of sodium fluoride were in low percentage in these three organs. On the contrary to these results, the inhibitory effects in the liver were 22.8−43.3 % by protamine sulfate, 14.5−32.1 % by sodium chloride and 63.5−70.2 % by sodium fluoride.

5) Alteration of the lipolytic activity after carbon tetrachloride poisoning.

At sixteen hours after carbon tetrachloride poisoning, they were sacrificed, and the LA of each organ was measured. These results are given in Table 5. The lipolytic activities in the adipose tissue, lung and heart were significantly increased ($P<0.05-0.01$), but those in the damaged liver and kidney did not show any remarkable change.

Table 5.
Lipolytic Activity in Organs from Carbon Tetrachloride Poisoned Subjects.

| Organ | Lung | Liver | Heart | Adipose tissue | Kidney |
|---|---|---|---|---|---|
| No. 1 | 530 | 304 | 632 | 574 | 214 |
| 2 | 483 | 269 | 596 | 820 | 292 |
| 3 | 516 | 280 | 664 | 615 | 244 |
| 4 | 612 | 252 | 596 | 607 | 272 |
| 5 | 456 | 315 | 581 | 680 | 289 |
| 6 | 486 | 290 | 608 | 582 | 306 |
| 7 | 442 | 353 | 544 | 746 | 258 |
| 8 | 435 | 308 | 589 | 705 | 278 |
| Mean value | 497 | 298 | 612 | 668 | 268* |
| ± S. D. | ±58 | ±44 | ±27 | ±78 | ±27 |

* Lipolytic Activity is expressed as mμ moles/tissue protein 1 mg/hour.

For the purpose to find the alteration of the LPL activity itself, the inhibitory effects by protamine sulfate, sodium chloride and sodium fluoride were measured, and these results are summarized in Table 6. The inhibitory effects were 63.4−71.0% by protamine sulfate and 52.5 −60.3 % by sodium fluoride in the lung, 67.0−76.6 % by protamine sulfate and 49.3−53.0 % sodium fluoride in the adipose tissue, 75.0− 81.4 % by protamine sulfate and 27.2−42.0 % by sodium fluoride in the heart, 40.3−55.6 % by protamine sulfate and 30.6−42.2 % by sodium fluoride in the kidney.

A remarkable change was found in the inhibitory effect in adipose tissue by sodium fluoride ($P<0.01$). The increment of the lipolytic

Table 6.
Effects of the Various Inhibitors on the Lipolytic Activity after Carbon Tetrachloride Poisoning.

| Organ | No. of Samples | Inhibitors | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Protamine Sulfate (20mg) | | | Sodium Chloried (1M) | | | Sodium Fluoride (0.2M) | | |
| | | Added | None | Inhibitory Effect in % | Added | None | Inhibitory Effect in % | Added | None | Inhibitory Effect in % |
| Lung | 3 | (173 | 516 | 66.5 | (126 | 516 | 75.6 | (299 | 516 | 42.0 |
| Mean value | | (167 | 456 | 63.4 | (190 | 456 | 58.0 | (265 | 456 | 41.8 |
| ± S. D. | | (143 | 487 | 71.0 | (163 | 487 | 66.3 | (296 | 487 | 39.2 |
| | | | | 67.0±3.1 | | | 66.6±7.2 | | | 41.0±1.3 |
| Liver | 3 | (182 | 280 | 35.0 | (172 | 280 | 38.8 | (133 | 280 | 52.5 |
| Mean value | | (234 | 314 | 25.6 | (185 | 314 | 41.2 | (144 | 314 | 54.5 |
| ± S. D. | | (154 | 291 | 47.0 | (161 | 291 | 44.6 | (115 | 291 | 60.3 |
| | | | | 35.8±8.8 | | | 41.5±2.4 | | | 55.8±3.4 |
| Heart | 3 | (175 | 702 | 75.0 | (183 | 702 | 74.0 | (511 | 702 | 27.2 |
| Mean value | | (109 | 585 | 81.4 | (136 | 585 | 77.0 | (339 | 580 | 42.0 |
| ± S. D. | | (114 | 570 | 79.5 | ( 94 | 570 | 83.5 | (343 | 570 | 39.7 |
| | | | | 78.6±2.7 | | | 78.3±4.0 | | | 36.4±6.5 |
| Adipose tissue | 3 | (266 | 615 | 76.6 | (254 | 615 | 58.6 | (312 | 615 | 49.3 |
| Mean value | | (209 | 680 | 69.0 | (221 | 680 | 67.5 | (322 | 680 | 53.0 |
| ± S. D. | | (193 | 582 | 67.0 | (213 | 582 | 63.4 | (287 | 582 | 50.6 |
| | | | | 71.0±4.1 | | | 63.1±3.6 | | | 51.0±1.5 |
| Kidney | 3 | (146 | 244 | 40.3 | (136 | 244 | 44.5 | (170 | 244 | 30.6 |
| Mean value | | (129 | 289 | 55.3 | (146 | 289 | 50.6 | (184 | 289 | 36.5 |
| ± S. D. | | (136 | 306 | 55.6 | (129 | 306 | 57.8 | (177 | 306 | 42.2 |
| | | | | 50.5±7.2 | | | 51.0± 5.4 | | | 36.5±4.7 |

♯ LA is expressed as mμ moles/tissue protein 1 mg/hour.

activity in the adipose tissue would be owing to the lipase which is inhibited by sodium fluoride.

6) Alteration of the lipolytic activity after administration of carbon tetrachloride and olive oil.

Table 7.
Lipolytic Activity after force-feeding of Carbon Tetrachloride and Olive Oil Mixture by Stomach Tube.

| Organ | Lung | Liver | Heart | Adipose tissue | Kidney |
|---|---|---|---|---|---|
| No. 1 | 562 | 269 | 740 | 635 | 255 |
| 2 | 594 | 298 | 792 | 812 | 272 |
| 3 | 615 | 308 | 736 | 672 | 238 |
| 4 | 555 | 322 | 710 | 820 | 245 |
| Mean value | 581 | 301 | 745 | 735 | 251* |
| ± S. D. | ±27 | ±21 | ±31 | ±15 | ±14 |

* Lipolytic Activity is expressed as mμ moles/tissue protein 1 mg/hour.

At sixteen hours after administration of carbon tetrachloride and olive oil mixture to the rats, they were sacrificed and the LA of each organ was measured. The results are given in Table 7.

The increase of the LA in the lung were somewhat higher than those only carbon tetrachloride was administered. There was no change on the LA in the liver. The increase of the LA in the adipose tissue was almost in same degree, and in the heart was higher than those only carbon tetrachloride was administered ($P<0.01$).

7) Alteration of the lipolytic activity after aseptic pneumonia.

Twenty-four hours after aseptic pneumonia was induced by silver nitrate solution administration in rats, they were sacrificed and the LA of each organ were determined. The results are summarized in Table 8.

Table 8.
Lipolytic Activity after Aseptic Pneumonia which was caused by Silver Nitrate Injection.

| Organ | Lung | Liver | Heart | Adipose tissue | Kidney |
|---|---|---|---|---|---|
| No. 1 | 589 | 273 | 687 | 704 | 242 |
| 2 | 532 | 248 | 733 | 646 | 230 |
| 3 | 623 | 285 | 641 | 765 | 251 |
| 4 | 504 | 232 | 706 | 605 | 273 |
| 5 | 532 | 266 | 725 | 776 | 254 |
| Mean value | 556 | 260 | 699 | 700 | 251* |
| ± S. D. | ±42 | ±19 | ±34 | ±65 | ±15 |

* Lipolytic Activity is expressed as mμ moles/tissue protein 1 mg/hour

In the lung tissue and also in the adipose tissue, the lipolytic activities were increased ($P<0.01$), similar to those of the carbon tetrachloride administration. There were no remarkable change in the liver and kidney. In the heart tissue, the LA was somewhat increased ($P<0.01$).

In this experiment, the inhibitory effects were 57.0－67.1% by protamine sulfate and 38.8－49.4% by sodium fluoride in the lung, 31.8－44.0% by protamine sulfate and 45.4－64.0% sodium fluoride in the liver, 53.0－77.1% by protamine sulfate and 42.6－56.4% by sodium fluoride in the adipose tissue, 69.8－80.5% by protamine sulfate and 33.8－45.4% by sodium fluoride in the heart, 52.6－60.0% by protamine sulfate and 34.2－42.5% by sodium fluoride in the kidney.

The inhibitory effects in the adipose tissue by sodium fluoride increasen significantly ($P<0.01$) similar to the carbon tetrachloride poisoning. (Table 9)

Table 9.
Effects of the Various Inhibitors on the Lipolytic Activity after Aseptic Pneumonia

| Organ | No. of Samples | Inhibitors | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Protamine Sulfate (20mg) | | | Sodium Chloride (1M) | | | Sodium Fluoride (0.2M) | | |
| | | Added | None | Inhibitory Effect in % | Added | None | Inhibitory Effect in % | Added | None | Inhibitory Effect in % |
| Lung | 3 | (217 | 589 | 63.1% | (189 | 589 | 68.0% | (298 | 589 | 49.4% |
| Mean value | | (175 | 532 | 67.1 | (214 | 532 | 59.9 | (325 | 532 | 38.8 |
| ± S. D. | | (217 | 504 | 57.0 | (186 | 504 | 63.0 | (301 | 504 | 42.0 |
| | | | | 62.5±4.2 | | | 63.6±3.3 | | | 43.5± 4.4 |
| Liver | 3 | (186 | 272 | 31.8 | (201 | 272 | 26.1 | (149 | 272 | 45.4 |
| Mean value | | (164 | 248 | 33.8 | (161 | 248 | 35.0 | ( 90 | 248 | 62.6 |
| ± S. D. | | (130 | 232 | 44.0 | (155 | 232 | 33.4 | ( 84 | 232 | 64.0 |
| | | | | 36.5±5.4 | | | 31.4±3.9 | | | 52.0±10.0 |
| Heart | 3 | (149 | 688 | 78.4 | (122 | 688 | 82.1 | (428 | 688 | 37.8 |
| Mean value | | (222 | 734 | 69.8 | (149 | 734 | 79.6 | (485 | 734 | 33.8 |
| ± S. D. | | (137 | 707 | 80.5 | (156 | 707 | 77.9 | (386 | 707 | 45.4 |
| | | | | 76.4±4.6 | | | 80.2±1.8 | | | 39.0± 4.8 |
| Adipose tissue | 3 | (331 | 704 | 53.0 | (184 | 704 | 74.0 | (307 | 704 | 56.4 |
| Mean value | | (147 | 646 | 77.1 | (240 | 646 | 68.5 | (323 | 646 | 50.0 |
| ± S. D. | | (216 | 605 | 64.0 | (151 | 605 | 75.0 | (348 | 605 | 42.6 |
| | | | | 64.8±9.8 | | | 72.5±2.9 | | | 49.7± 5.6 |
| Kidney | 3 | ( 97 | 242 | 60.0 | (115 | 242 | 52.5 | (139 | 242 | 42.5 |
| Mean value | | (109 | 230 | 52.6 | ( 85 | 230 | 63.1 | (151 | 230 | 34.2 |
| | | (124 | 273 | 54.5 | (121 | 273 | 44.4 | (161 | 273 | 41.2 |
| | | | | 55.7±3.1 | | | 53.4±7.7 | | | 39.3± 3.6 |

# LA is expressed as mμ moles/tissue protein 1 mg/hour.

## DISCUSSION

Since Hahn had observed the clearing phenomen of lipemic plasma after heparin administration, it was accepted by many investigators[25)26)27)] that a lipolytic or lipemia clearing enzyme appears in plasma soon after intravenous injection of heparin, though this enzyme was not yet shown in pure form.

Thereafter E.D. KORN[28)], J.I. KESSLER[29)], M. FINKEL, D.S. ROBINSON[30)], G. ANGERVALL, A. DURY[31)] and J. SLACK[32)] have observed that the activity of lipoprotein lipase were in the heart, aorta, adipose tissue, pancreas and mammary gland.

D.S. ROBINSON and J.E. FRENCH[33)] suggested about the relation between LPL in plasma and tissue that LPL occurs on the capillary surface and the role of heparin would be to displace and release this enzyme into the blood stream. Furthermore, D.S. ROBINSON and P.M. HARRIS[34)35)] suggested that the vascular component having responsibility for the production of LPL might be distributed generally in the walls of vessels.

In this study, it was shown that the heart and the adipose tissue have high lipolytic activity by means of the improved assay system, as in the leading investigators have reported. However, it was also shown that the lung has high lipolytic activity which was approximately the same as in heart and adipose tissue.

These results would be very important as a key to study the relationship between the lung and neutral lipid metabolism.

Among the lipolytic activity of each organ after administration of fat, the lipolytic activity in lung was increased as high as in the heart and the LA tend to elevate after fat ingestion, except it in liver. Most remarkable changes were noted in adipose tissue, lung and heart.

However, the addition of heparin into assay system elevated the LA of lung and heart, but did not change significantly in adipose tissue. The elevation of the LA was more marked in the lung than in the heart.

J. I. KESSLER[36] reported that post-Lipomul lipolytic activity was essentially similar to those of post-heparin plasma and H. ENGELBERG[37] demonstrated that after infusion of Lipomul, endogeneous heparin increased in blood stream.

These facts are sufficient to assume that chylomicrons have caused the production or the release of heparin and the secondary production of LPL.

The results, obtained in this study, support this aspect, and also suggest that the lung would be the main organ to produce LPL, because the addition of heparin in vitro caused to elevate the LA remarkably mainly in lung. The liver is thought to be only a site of destruction and it is reasonable that the liver did not show any change.

HAVEL et al[38] observed in the tracer experiment that 1/3 of the exogeneous lipid, which was as chylomicron in blood, was taken up in liver by means of pynocytosis and 2/3 of that in extrahepatic cells by means of LPL, although how LPL play a role to take up the triglyceride in extrahepatic cells is quite unknown.

Amoung the extrahepatic cells, the attitude of LPL in adipose tissue would have something different to those of lung and heart, because addition of heparin in vitro did not show any effect.

The lipolytic activity was not only shown by the LPL but also by the pancreatic lipase, because the assay system does not detect completely the LPL alone. The present results on various inhibitors demonstrate these facts.

The proportion of LPL inhibited by protamine sulfate, to pancreatic lipase (PL) inhibited by sodium fluoride, were similar in all organ except liver. It was observed in the liver damaged group and the aseptic pneumonia group, that the lipolytic activity in adipose tissue, lung and heart were elevated in comparing with the intact group,

however the proportion of LPL to PL in LA of lung and heart were mostly similar to that in intact group and those in LA of adipose tissue were changed to the increase of PL in both group compared with intact group.

Although the disturbance of lipid metabolism in aseptic pneumonia is quite unknown, there were many studies on the fatty liver induced by carbon tetrachloride poisoning. D. N. CARBERT and T. M. BRODY[39)40)] proposed an hypothesis that the characteristic hepatic change by carbon tetrachloride poisoning were the results of a stimulation of the sympathetic nervous system, which caused the restriction of blood flow in liver, and finally let it to necrosis around the central vein of the lobule, and also which caused to increase the release of NEFA from the peripheral fat depot.

R. O. RECKNAGEL, B. LOMBARDI and M. C. SCHOTZ[41)] recently presented the hypothesis, that the liver is constantly secreting a large quantities of triglyceride into the blood stream and the carbon tetrachloride would blockade this secretory mechanism.

INOUE et al[42)] reported from the other point of view that the fatty acid pattern in the fatty liver, induced by carbon tetrachloride injection, were almost the same as that in adipose tissue.

From these hypothesis and results it could be supposed that the lipid flows mainly from adipose tissue to liver in carbon tetrachloride poisoned animals.

From the above mentioned it would be reasonable to consider the LA in the adipose tissue increase to release NEFA.

Although why the LA in lung and heart were increased in these instances are unknown, it would be very favorable to understand that LPL play a role to take up the triglyceride and PL to release the NEFA. Aseptic pneumonia also demonstrated almost similar pattern in the inhibitory study and the LA activity to those in carbon tetrachloride poisoning.

These results showed that the dynamic state of lipid in aseptic pneumonia were severe as it in fatty liver.

Finally, it is very difficult to explain clearly the mechanism of the lipid metabolism disturbance in aseptic pneumonia, however these experiments show the important role of the lung in neutral lipid transportation.

Acknowledgement.

The auther is grateful to Prof. Shiro OSAJIMA, Mitsuji INOUE M. D. and Noritaka WATADA M. D. for their continuous helpful advices and encouragements.

## REFERENCES


1) Hahn P. F. : *Science*, 98: 19, 1943.
2) Anderson N. G. and Fawcatt B. : *Proc. Soc. Exp. Biol. and Med.*, 74 : 768, 1950.
3) Graham D. M. et al : *Circulation* 4 : 666, 1951.
4) Kern F. Jr., Steimann L. and Sanders B. B. : *J. Lipid Research*, 2 (1) : 51, 1961.
5) Takenaka T. : *Japanese Circulation Journal*, 23 (6) : 802, 1959 (Japanese).
6) Conner W. E. : *Proc. Soc. Exp. Biol. and Med.*, 106 (2) : 378, 1961.
7) Constantinides P., So Y. and Johnstone F. R. C. : *Proc. Soc. Exp. Biol. and Med.*, 100 : 262, 1959.
8) Hollenberg C. H. : *Am. J. Physiol.*, 197 : 667, 1959.
9) Cherkes A. and Gordon R. S. Jr. : *J. Lipid Research* 1 (1) : 97, 1960.
10) Gordon R. S. Jr. and Cherks A. : *Proc. Soc. Exp. Biol. and Med.*, 107 : 853, 1961.
11) Carlson L. A. : *Acta Medica Scandinavica*, 173 : 6, 1963.
12) Korn E. D. : *J. Biol. Chem.*, 215 (1) : 15, 1955.
13) Dury A. : *Proc. Soc. Exp, Biol. and Med.*, 107: 299, 1961.
14) Nakatani M., Nakamura M. and Torii S. : *Proc. Soc. Exp. Biol. and Med.*, 107 : 853, 1961.
15) Frederickson D. S. and Gordon R. S. Jr. : *Physiol. Revs.*, 38 : 585, 1958.
16) Robinson D. S. : *Am. J. Clin. Nutrition*, 8 : 7, 1960.
17) Matsuo H. : *Nipponrinsho*, 17 (4) : 122, 1959 (Japanese).
18) Nakagawa S. : *The Journal of Japanese Society of Internal Medicine*, 50 (11) : 1206, 1962 (Japanese).
19) Osajima S. et al : *The Journal of Japanese Society of Internal Medicine*, 48 (9) : 1414, 1960 (Japanese).
20) Dole V. P. : *J. Clin. Invest.*, 35 : 150, 1956.
21) Angervall G. and Hood B. : *Acta Medica Scandinavica*, 157 (5) :407, 1957.
22) Angervall G. : *Acta Physiol. Scand.*, 40 : 71, 1960.
23) Hood B. and Angervall G. : *Acta Medica Scandinavica*, 158 (1) : 13, 1957.
24) Monkhouse F. C. and MacKneson R. G. : *Can. J. Biochem. Physiol.*, 36 : 1065, 1958.
25) Datta D. V. : *Porc. Soc. Exp. Biol. and Med.*, 112 (4) : 1006, 1963.
26) Okaniwa H. : *Nissinigaku*, 48 (11) : 730, 1960 (Japanese).
27) Yoshitoshi Y., Naito C., Okaniwa H., Usui M. Mogami T. and Tomono T. : *J. Clin. Invest.*, 42 (5) : 707, 1963.
28) Mc. Bride O. W. and Korn E. D. : *J. Lipid Research*, 4 (1) : 17, 1960.
29) Kessler J. I., Finkel M., Dreiling D. A. and Ganowitz H. D. : *Proc. Soc. Exp. Biol. and Med.*, 113 (1) : 127, 1963.
30) Robinson D. S. : *J. Lipid Research*, 4 (1) : 21, 1963.
31) Dury A. : *J. Gelontology*, 16 (2) : 114, 1961.
32) Slack J. et al : *J. Lab. & Clin. Med.*, 59 (2) : 302, 1961.
33) Robinson D. S. and French J. E. : *Quart. J. Exp. Physiol.*, 42 : 151, 1957.
34) Robinson D. S. and Harris P. M. : *Quart. J. Exp Physiol.*, 44 : 80, 1959.
35) LEQuire V.S. : *Proc. Soc. Exp. Biol. and Med.*, 114 (1) : 104, 1963.
36) Kesser J. I. : *J. Lab. Clin. Med.*, 59 (4) : 558, 1962.
37) Engelberg H. : *Proc. Soc. Exp. Biol. and Med.*, 97 : 304, 1958.
38) Nestle R. J., Havel R. J. and Bezman A. : *J. Clin. Invest.*, 42 : 1313, 1963.
39) Carbert D. N. and Brody T. M. : *Am. J. Physiol.*, 198 (3) : 669, 1960.
40) Brody T. M. and Carbert D. N. : *Am. J. Physiol.*, 198 : 982, 1960.
41) Recknagel R. O., Lombardi B. and Schetz M. C. : *Proc. Soc. Exp. Biol. and Med.*, 104 : 608, 1960.
42) Inoue M., Sumida T. and Kikitsu Y. *Medicine and Biology*, 65 (2) : 34, 1962 (Japanese).